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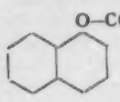
Pharmacological Effects of Carbaryl II. Modification of Serotonin Metabolism in the Rat Brain

In a previous paper[1] it was reported that the administration of a mixture of DDT, parathion and carbaryl (1-naphthyl-N-methylcarbamate) induced an increased elimination of urinary 3-methoxy-4-hydroxy-mandelic acid (VMA) and 5-hydroxy-3-indolylacetic acid (5-HIAA) in the rabbit. It was postulated that a stress mechanism is involved, featuring an increased synthesis rate of the catecholamines and Serotonin respectively. HASSAN[2] demonstrated an increased sympathoadrenergic activity in the rat, following the oral administration of carbaryl. This increased activity involved increased synthesis of norepinephrine periferally (and probably centrally) with concomitant increase of urinary VMA excretion.



(Carbaryl)

The purpose of the present investigation was to study the effect of a single carbaryl dose on serotonin metabolism in the rat brain.

Male albino rats (Holtzman) weighing 150–180 g were used in this study. All animals were housed in groups for several weeks, and fed a standard diet of Purina laboratory chow. Carbaryl was administered orally as a suspension in peanut oil at a single dosage of 60 mg/kg. Control rats received only peanut oil. Animals were killed by decapitation, and the brain was quickly removed and extracted. All determinations were made on whole brain. Serotonin was determined by the procedure of BOGDANSKI[3] and 5-HIAA by the method of GIACALONE and VALZELLI[4]. The concentration of corticosterone in the plasma was estimated by a fluorescence method[5]. The Aminco Bowman Spectrophotofluorometer was used for all fluorescent measurements.

The results of the study on the level of 5-HT and 5-HIAA in the brain are shown in the Table. The concentration of the amine and its metabolite increased significantly after 2, 4 and 6 h following carbaryl administration. After 24 h the level of both substances returned to normal.

In order to investigate the effect of carbaryl on brain 5-HT formation, endogenous stores of the amine were previously depleted by inhibition of its synthesis. Carbaryl was given 72 h after *p*-chlorophenylalanine (PCPA) administration and animals were sacrificed 4 h later. The amine level was significantly higher when compared with PCPA control value (Figure 1). Reserpine-pretreated animals showed the same trend. Plasma corticosterone level was also increased by about 125%, one hour after the oral administration of the carbamate (Figure 2).

[1] A. HASSAN and C. CUETO JR., Z. Naturforsch. *25b*, 521 (1970).
[2] A. HASSAN, Biochem. Pharmac. (1971).
[3] D. F. BOGDANSKI, A. PLETSCHER, B. B. BRODIE and S. UDENFRIEND, J. Pharmac. exp. Ther. *117*, 82 (1956).
[4] E. GIACALONE and L. VALZELLI, J. Neurochem. *13*, 1265 (1966).
[5] R. H. SILBER, R. D. BUSH and R. OSLAPAS, Clin. Chem. *4*, 278 (1958).

Effect of a single carbaryl dose on brain 5-HT and 5-HIAA

Hours after Carbaryl	5-HT		5-HIAA		Ratio 5-HIAA 5-HT
	Concentration μg/g ± S.E.	% Increase	Concentration μg/g ± S.E.	% Increase	
0	0.61 ± 0.05	–	0.25 ± 0.02	–	0.41
2	0.78 ± 0.07	28	0.30 ± 0.02	20	0.38
4	0.78 ± 0.08	28	0.31 ± 0.03	24	0.40
6	0.80 ± 0.07	31	0.31 ± 0.03	24	0.39
24	0.60 ± 0.06	–	0.24 ± 0.02	–	0.40

Carbaryl dose 60 mg/kg. Results are mean of groups of 5–8 animals ± S.E.

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This probably represents the maximum elevation of the glucocorticoid. The level drops thereafter and approached pretreatment levels after 20 h. In control experiments, the administration of peanut oil alone did not cause an increase in the plasma corticosterone, after 1 h.

The effect of carbaryl on cerebral serotonin metabolism is believed to be related to a 'stress mechanism'. The effect simulates that of electric stress[6], and is presumably provoked by an enhanced synthesis and not by a decreased utilization of the amine. This assumption is supported by some complimentary evidence. 1. Serotonin level in the brain of carbaryl-treated animals, pretreated with PCPA was 72% higher than their respective controls. These results suggest that 5-HT synthesis is accelerated by carbaryl. The possibility that the effect of PCPA is diminished by carbaryl cannot be ruled out. 2. The depletion of brain 5-HT by reserpine could also be partially antagonized by carbaryl. 3. The formation of higher amounts of 5-HIAA in the brain is probably a consequence of an increased synthesis rate of serotonin, and may represent a higher functional utilization of the amine. Since organic acids are removed from the brain by an active energy-requiring process[7], an effect on transport by carbaryl could contribute to the changes in cerebral 5-HIAA levels observed.

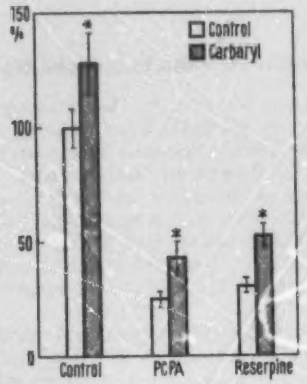

Fig. 1. Effect of carbaryl on endogenous 5-HT levels in the brain of normal and PCPA or reserpine-pretreated animals. PCPA pretreated animals received the drug i.p. (360 mg/kg) 72 h before administration of carbaryl and animals were killed 4 h later. Reserpine (5 mg/kg) was administered i.p., immediately before carbaryl was given, animals were killed 4 h later. Results are expressed as a percentage of control values ± S.E. The endogenous 5-HT levels in control animals were 0.61 ± 0.05 µg/g. * $P < 0.01$ when compared with respective controls.

The increase in the biosynthetic rate of serotonin may involve activation of tryptophan hydroxylase and/or hydroxytryptophan decarboxylase. The possible activation of tryptophan hydroxylase receives support from the PCPA experiments. The inhibiting power of PCPA of tryptophan hydroxylase[8] could be – at least partially – overcome by carbaryl. It is also worth mentioning that stress induced by acute cold exposure significantly increases cerebral tryptophan-5-hydroxylase activity[9].

The changes in the brain amine level unlikely involve monoamine oxidase inhibition, because of the concomitant increase in cerebral 5-HIAA. In fact the ratio 5-HIAA/5-HT remained essentially unaltered (no significant differences) (Table). The administration of carbaryl into rats was reported to increase the degradation of catecholamines, presumably through activation of monoamine oxidase[8]. This suggests in turn that the increased formation of cerebral 5-HIAA in carbaryl-treated rats is probably the result of MAO activation.

The administration of carbaryl also strongly stimulates corticosterone secretion by the adrenals. Normally, corticosterone secretion would be considered an index of the release of ACTH. However, it is not known whether the changes of serotonin metabolism are related to the activation of the pituitary-adrenal system. Under conditions of restraint stress, brain 5-HT level did not correlate with the adrenocortical activation[10].

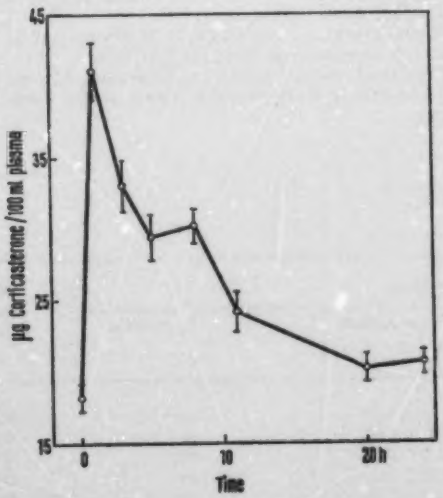

Fig. 2. Effect of a single carbaryl dose (60 mg/kg) on plasma corticosterone. Points represent means of at least 5 animals and bars indicate ± S.E.

Zusammenfassung. Nachweis, dass Carbaryl die Konzentration von Serotonin und 5-Hydroxyindolessigsäure im Rattenhirn steigert. Der Zusammenhang mit einem Stress wird als möglich angenommen.

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[6] A. M. Thierry, M. Fekete and J. Glowinski, Europ. J. Pharmac. *4*, 384 (1968).
[7] B. Werdinius, J. Pharm. Pharmac. *18*, 546 (1966).
[8] E. Jéquier, W. Lovenberg and A. Sjoerdsma, Molec. Pharmac. *3*, 274 (1967).
[9] E. M. Gal, R. D. Heater and S. A. Millard, Proc. Soc. exp. Biol. Med. *128*, 412 (1968).
[10] A. De Schaepdryver, P. Preziosi and U. Scapagnini, Br. J. Pharmac. *35*, 460 (1969).
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